

FIGURE 1

Human G Protein Coupled Receptor Family

(Receptors known as of January, 1999)

CLASS	LIGAND	NUMBER	TISSUE	PHYSIOLOGY	THERAPEUTICS
•Class I Rhodopsin like	•Amine				
	•Acetylcholine (muscarinic & nicotinic)	5	Brain, Nerves, Heart	Neurotransmitter	Acuty, Alzheimer's
	•Adrenoceptors				
	•Alpha Adrenoceptors	6	Brain, Kidney, Lung	Gluconeogenesis	Diabetes, Cardiovascular
	•Beta Adrenoceptors	3	Kidney, Heart	Muscle Contraction	Cardiovascular, Respiratory
	•Dopamine	5	Brain, Kidney, GI	Neurotransmitter	Cardiovascular, Parkinson's
	•Histamine	2	Vascular, Heart, Brain	Vascular Permeability	Anti-inflammatory, Ulcers
	•Serotonin (5-HT)	16	Most Tissues	Neurotransmitter	Depression, Insomnia, Analgesic
	•Peptide				
	•Angiotensin	2	Vascular, Liver, Kidney	Vasoconstriction	Cardiovascular, Endocrine
	•Bradykinin	1	Liver, Blood	Vasodilation,	Anti-inflammatory, Asthma
	•C5a anaphylatoxin	1	Blood	Immune System	Anti-inflammatory
	•Forsk-leu-ple	3	Blood	Chemoattractant	Anti-inflammatory
	•Interleukin-8	1	Blood	Chemoattractant	Anti-inflammatory
	•Chemoikine	6	Blood	Chemoattractant	Anti-inflammatory
	•Orexin	2	Brain	Fat Metabolism	Obesity
	•Nociceptin	1	Brain	Bronchodilator, Pain	Airway Diseases, Anesthetic
	•CCK (Gastrin)	2	Gastrointestinal	Motility, Fat Absorption	Gastrointestinal, Obesity, Parkinson's
	•Endothelin	2	Heart, Bronchus, Brain	Muscle Contraction	Gastrointestinal, Respiratory
	•Melanocortin	5	Kidney, Brain	Metabolic Regulation	Cardiovascular, Analgesics
	•Neuropeptide Y	1	Nerves, Intestine, Blood	Neurotransmitter	Behavior, Memory, Cardiovascular
	•Neurotensin	5	Brain,	CNS	Cardiovascular, Analgesic
	•Opioid	3	Brain,	CNS	Depression, Analgesic
	•Somatostatin	5	Brain, Gastrointestinal	Neurotransmitter	Oncology, Alzheimer's
	•Tachykinin (Substance P, NKA ₁)	3	Brain Nerves	Neurohormone	Depression, Analgesic

•Thrombin	3	Platelets, Blood Vessels	Coagulation	Anti-coagulant, Anti-inflammatory
•Vasopressin-like	4	Arteries, Heart, Bladder	Water Balance	Anti-diuretic, Diabetic Complications
•Galatin	1	Brain, Pancreas	Neurotransmitter	Analgesics, Alzheimer's
•Hormone protein	1	Ovary, Testis	Endocrine	Infertility
•Follicle stimulating hormone	1	Ovary, Testis	Endocrine	Infertility
•Lutropin-choriogonadotropin	1	Thyroid	Endocrine	Thyroidism, Metabolism
•Thyrotropin	1			
•Rhodopsin	5	Eye	Photoreception	Ophthalmic Diseases
•Opsin	4 (~1000)	Nose	Smell	Olfactory Diseases
•Olfactory				
•Prostanoid	5	Arterial, Gastrointestinal	Vasodilation, Pain	Cardiovascular, Analgesic
•Prostaglandin	2	Vessels, Heart, Lung	Inflammation	Cancer, Anti-inflammatory
•Lysophosphatidic Acid	2	Most Cells	Cell proliferation	Cancer
•Sphingosine-1-phosphate	1	White Blood Cells, Bronchus	Inflammation	Asthma, Rheumatoid Arthritis
•Leukotriene	1	Arterial, Gastrointestinal	Platelet Regulation	Cardiovascular
•Prostacyclin	1	Arterial, Bronchus	Vasoconstriction	Cardiovascular, Respiratory
•Thromboxane	1			
•Nucleotide-like	4	Vascular, Bronchus	Multiple Effects	Cardiovascular, Respiratory
•Adenosine	4	Vascular, Platelets	Relaxes Muscle	Cardiovascular, Respiratory
•Purinoreceptors	2	Brain	Sensory Perception	Analgesics, Memory
•Cannabis	1	Most Peripheral Tissues	Inflammation	Anti-inflammatory, Anti-asthmatic
•Platelet activating factor				
•Gonadotropin-releasing hormone like	1			
•Gonadotropin-releasing hormone	1	Reproductive Organs, Pituitary	Reproduction	Prostate Cancer, Endometriosis
•Thyrotropin-releasing hormone	1	Pituitary, Brain	Thyroid Regulation	Metabolic Regulation
•Growth hormone-inhibiting factor	1	Gastrointestinal	Neuroendocrine	Oncology, Alzheimer's
•Melatonin	1	Brain, Eye, Pituitary	Neuroendocrine	Regulation of Circadian Cycle
•Secretin	1	Gastrointestinal, Heart	Digestion	Obesity, Gastrointestinal
•Calcitonin	1	Bone, Brain	Calcium Resorption	Osteoporosis
•Corticotropin releasing factor/urocortin	1	Adrenal, Vascular, Brain	Neuroendocrine	Stress, Mood, Obesity
•Gastric inhibitory peptide (GIP)	1	Adrenals, Fat Cells	Sugar/Fat Metabolism	Diabetes, Obesity
•Glucagon	1	Liver, Fat Cells, Heart	Gluconeogenesis	Cardiovascular

•Class II
 Secretin like

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●ClassIII	•Glucagon-like Peptide 1 (GLP-1)	1	Pancreas, Stomach, Lung	Glucogenesis	Cardiovascular, Diabetes, Obesity
	•Growth hormone-releasing hormone	1	Brain	Neuroendocrine	Growth Regulation
	•Parathyroid hormone	1	Bone, Kidney	Calcium Regulation	Osteoporosis
	•PACAP	1	Brain, Pancreas, Adrenals	Metabolism	Metabolic Regulation
	•Vasoactive intestinal polypeptide (VIP)	1	Gastrointestinal	Motility	Gastrointestinal
	•Metabotropic Glutamate	7	Brain	Sensory Perception	Hearing, Vision
	•GABA _B	1	Brain	Neurotransmitter	Mood Disorders
	•Extracellular Calcium Sensing	1	Parathyroid, Kidney, GI Tract	Calcium Regulation	Cataracts, GI Tumors

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Figure 2

G protein-coupled receptors:

(Division into Class A

Or Class B)

1. **A1 adenosine receptor** [Homo sapiens]. ACCESSION AAB25533
npivayf riqkfrvtfl kiwndhfrcq pappidedlp eerdd
Class A
2. **adrenergic, alpha -1B-, receptor** [Homo sapiens]. ACCESSION NP_000670
npiityc sskefkrafv rilgcqcrgr grrrrrrr lggcaytyrp wtrggslers qsrkdsldds gscslgsqrt
lpsaspspgy lrggapppe lcafepwkap gallspape ppgrrgrhds gplftklft epespgtdgg asnggceaaa
dvangpggfk snmplapgqf
Class A
3. **adrenergic receptor alpha-2A** [Homo sapiens]. ACCESSION AAG00447
npviytifn hdfirafkki lcrgrkriv
Class A
4. **alpha-2B-adrenergic receptor** - human. ACCESSION A37223
npviytifn qdfirafri lerpwtqtaw
Class A
5. **alpha-2C-adrenergic receptor** - human. ACCESSION A31237
npviytfv qdfirafkhi lfrrrrgfr q
Class A
6. **beta-1-adrenergic receptor** [Homo sapiens]. ACCESSION NP_000675
npiiyers pdfirafqgl lccarraarr rhathgdrpr asgclarpgp ppspgaaadd ddddvvgatp parllepwag
cnggaaadds ssldepcrgp faseskv
Class A
7. **beta-2 adrenergic receptor**. ACCESSION P07550
npiyersp dfriaqell clrrsslkay gngyssngnt 361 geqsyghveq ekenklced lpgtedfvgh qgtvpsdnid
sqgrncstnd sll
Class A
8. **dopamine receptor D1** [Homo sapiens]. ACCESSION NP_000785
npii yafnadfrka fstllgcyril cpatnnaiet vsinnngaam fssheprgs iskecnlvyl iphavgsedd
lkkeeaagia rpleklspal svildytdtv slekiquitq ngqhpt
Class A
9. **D(2) dopamine receptor**. ACCESSION P14416
npiiyttfn iefrakfli lhc
Class A

10. **d3 dopamine receptor** - human. ACCESSION G01977
np viyttfnief rkafilkisc
Class A
11. **dopamine receptor D4** - human. ACCESSION DYHUD4
npviyvtv fnaefmvfr kalracc
Class A
12. **dopamine receptor D5** - human. ACCESSION DYHUD5
npviya fnadfukvfa qligeshfcs rtpvetvnis nelisynqdi vfhkeiaaay ihmmpnavtp gnrevnddee
egpfdrmfqi yqtspgdgv aesvweldee geislkitp flpngfh
Class A
13. **muscarinic acetylcholine receptor M1** [Homo sapiens]. ACCESSION NP_000729
nrmcyal enkafrdtfr llllerwdkr rwrkiplrpg svhrtpsrgc
Class A
14. **muscarinic acetylcholine receptor M2** [Homo sapiens]. ACCESSION NP_000730
npacy alcnatfkkt fkhllmchyk nigatr
Class A
15. **muscarinic acetylcholine receptor M3** [Homo sapiens]. ACCESSION NP_000731
n pvcyalcnkt frttfkmlll cqedkkkrrk qqyqqrqsvi fhkrapeqal
Class A
16. **muscarinic acetylcholine receptor M4** [Homo sapiens]. ACCESSION NP_000732
npa cyalcnatfk ktfrhllecq yrnigtar
Class A
17. **m5 muscarinic receptor**, locus HUMACHRM ACCESSION AAA51569
npieyalcnr tfrktfkmll lcrwkkkkve eklywqgnsk lp
Class A
18. **5-hydroxytryptamine (serotonin) receptor 1A** [Homo sapiens]. ACCESSION BAA90449
npviy ayfinkdfqna fkkiikckf
Class A
19. **5-hydroxytryptamine (serotonin) receptor 1B** [Homo sapiens]. ACCESSION BAA94455
npiiyt msnedfkqaf hklirfkets
Class A
20. **5-hydroxytryptamine (serotonin) receptor 1E** [Homo sapiens]. ACCESSION BAA94458
n pllytsfined fklafkkir cre
Class A

21. **OLFACTORY RECEPTOR 6A1.** ACCESSION O95222
 npiiyelrmq evkralccil hlyqhqdppd kkgssrnv
Class A
22. **OLFACTORY RECEPTOR 2C1.** ACCESSION O95371
 npliyl tlrnmekga lrrllgkgre vg
Class A
23. **angiotensin receptor 1 [Homo sapiens].** ACCESSION NP_033611
 npl fyglgkklk ryflqlkyl ppkakshnl ~~sfkmsfl~~syrr psdvnvssstk kpapecfeve
Class B
24. **angiotensin receptor 2 [Homo sapiens].** ACCESSION NP_000677
 npflycf vgnrfqgklr svfrvpitwl qgkresmscr ~~ksssl~~remet fvs
Class B
25. **interleukin 8 receptor beta (CXCR2) [Homo sapiens].** ACCESSION NM_001557
 NPLIYAFIGQKFRHGLKLKLAHGLISKDSLFPKDSRPSFVGSSSGHTSTTL
Class B
26. **cx3c chemokine receptor 1 (cx3cr1) (fractalkine receptor)**
 ACCESSION P49238
 np liyafagekf rrylyhlyk clavlegrsv hvdffssscsq rsrhgsvlss nftyhtsdgd allll
Class B
27. **neurotensin receptor - human.** ACCESSION S29506
 n pilynlvsan frhiflatla clepvwrrrr krpafsrkad ~~svssnhflss~~ natretly
Class B
28. **SUBSTANCE-P RECEPTOR (SPR) (NK-1 RECEPTOR) (NK-1R).** ACCESSION P25103
 npiiyccldn rfrlgfkfah rccpfisagd yeglemkstr yltqgsvyk vsrleffstvtvgaheeepe dgpkatpssl
 dltsncssrs dskmtmtesfs fssnvl
Class B
29. **vasopressin receptor type 2 [Homo sapiens].** ACCESSION AAD16444
 npwiyasfss svsselsll ccargtrpps lgpqdcscff ~~assslakdts~~ s
Class B
30. **thyrotropin-releasing hormone receptor - human.** ACCESSION JN0708
 npviy nlmsqkfraa frklenckqk ptekpanysv alnysvikes dhfstelddi tvtdtlysat kvsfddtcla sevsfsqs
Class B
31. **oxytocin receptor - human.** ACCESSION A55493
 npwiyml lftghlflhel vqrflccsas ylkgrlget ~~saskksnsss~~ fvlshrsssq rscsqpsta
Class B

32. **neuromedin U receptor [Homo sapiens]**. ACCESSION AAG24793
npvylslmssrfretfgealcigacchrlrprhsshslsrmttgstlcdvsglsgwvhlplagndgeaqqetdps
Class B
33. **gastrin receptor**. ACCESSION AAC37528
nplvy cfmhrfrfqa cletcarccp rpprarpral pdedpppsi aslsrlsytt istlpgg
Class B
34. **galanin receptor 3 [Homo sapiens]**. ACCESSION 10879541
nplv yalasrhfra rfrlwpcgr rrrhraral rrvrpassgp pgcpgdarps grllagggqg pepregpvhg geaargpe
Class A
35. **edg-1 - human**. ACCESSION A35300
npitltnkemrra firimscccke psldsagkfk rpiiagmefsrksdnsshp 361 qkdegdnpet imssgnvnss
Class A
36. **central cannabinoid receptor [Homo sapiens]**. ACCESSION NP_057167
npitiyair skdlrlnafis nupscceglag pldnsmgdsd cllkharnaa svhraacesi kstvkikvlt msvsltdisae ai
Class A
37. **delta opioid receptor - human**. ACCESSION I38532
npvlyaf ldenfkrcfr qlerkpcgrp dpssfsrpre atarervtac tpsdpggggr aa
Class A
38. **proteinase activated receptor 2 (PAR-2) human**. ACCESSION P55085
dpfvyyfvshdfrdhaknallersvrtvkmqmvsltskkhsrksyssstttvktsty
Class A
39. **vasopressive intestinal peptide receptor (VIPR) rat**. ACCESSION NM_012685
NGEVQAE LRK WRR WHL QGV LGW SSK SQHPWGGSNGATCSTQV SML TRV SPSARR
SSSFQAEVSLV
Class B

Figure 3

A. Human V2R DNA (nucleotides encoding the last 29 amino acids of the V2R and the adjacent stop codon):

gccccgggagcgacccccaccagcctgggtcccaagatgagtctgcaccaccgccagtcct
ccctggccaaggacattcatcgtga

B. PCR amplified human V2R DNA fragment:

gcgccgcacgggagcgacccccaccagcctgggtcccaagatgagtctgcaccaccgc
agtcctccctggccaaggacattcatcgtgaagatctccgcggtctaga

*Additions and changes to the V2R DNA are underlined.

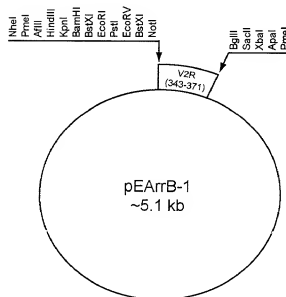
*The Sma I (ccggg) restriction enzyme site (underlined in Fig. 3A) was eliminated in the amplified DNA fragment by changing a cytosine to an adenine.

*A Not I restriction site (gcggccgc) was incorporated into the amplified DNA fragment by adding 6 nucleotides (gcggcc) to the 5' end of the V2R DNA.

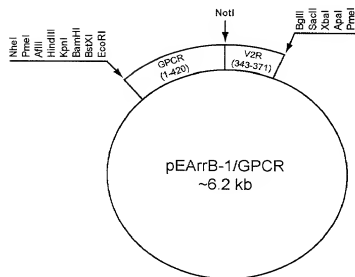
*Bgl II (agatct), Sac II (ccggg), and Xba I (tctaga) restriction enzyme sites were added to the 3' end of the V2R DNA.

Figure 4

A.



B.



C.

...AAARGRTPPSLGPQDESCTTASSSLAKDTSS

Figure 5

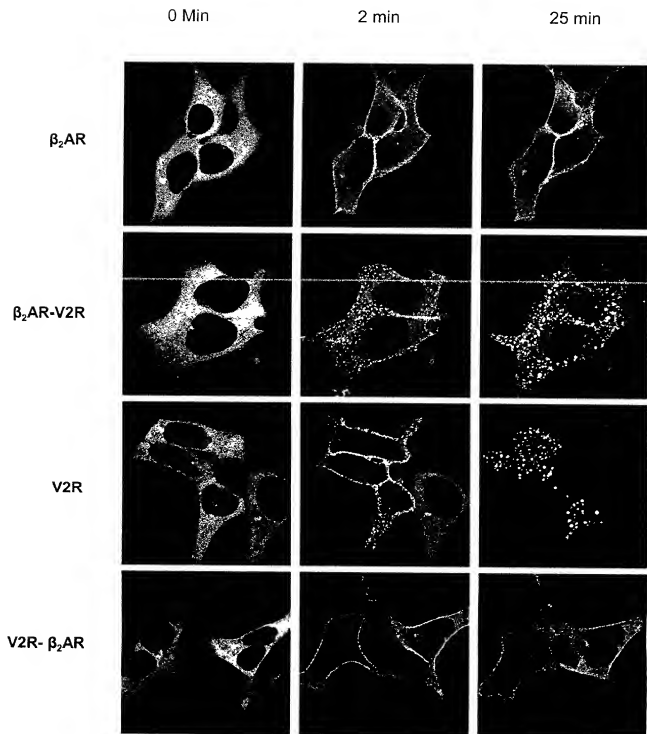


Figure 6

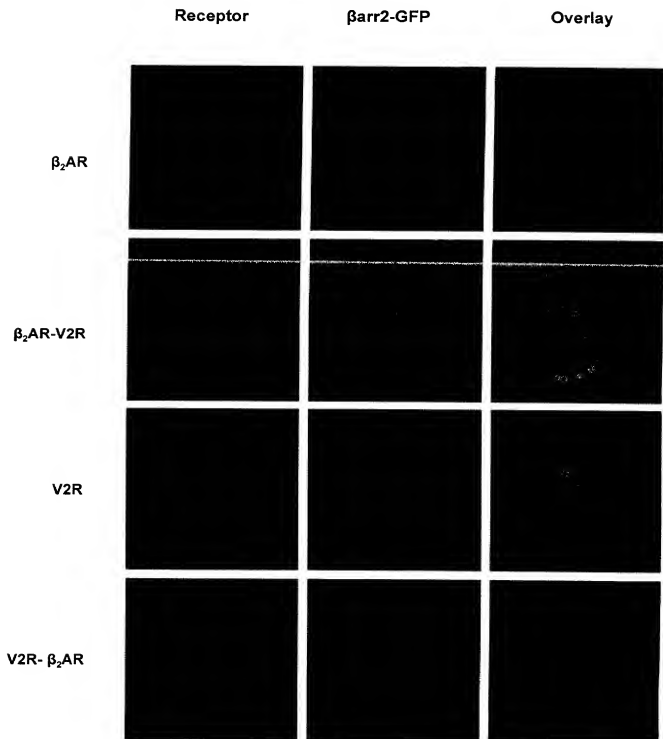


Figure 7

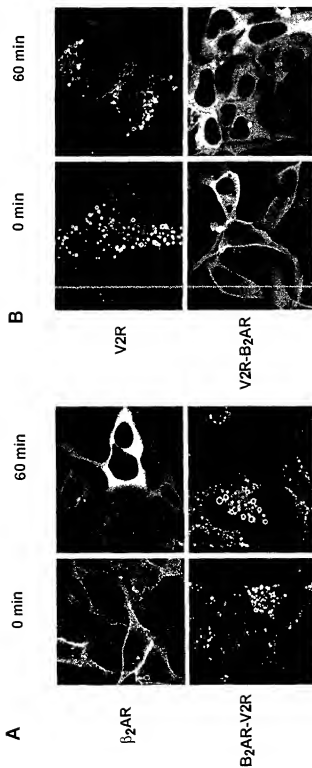


Figure 8

A

1) V2R	CARGRTPPSLGQDESCCTTASSSLAKDTSS
2) V2R-S362X	CARGRTPPSLGQDESCCTTA
3) V2R-S3STSS/AAAAAA	CARGRTPPSLGQDESCCTTAAALAKDAAA
4) V2R-TSS/AAA	CARGRTPPSLGQDESCCTTASSSLAKDAAA
5) V24-SSS/AAA	CARGRTPPSLGQDESCCTTAAALAKDTSS
6) β AR-V2R-SSS/AAA	CARGRTPPSLGQDESCCTTAAALAKDTSS
7) β AR	CLFRSSLKAYGNGYSSNGTGEQSGYHVEQEKENKLLCHDLP- GTEDFVGHGTVPSDNDIDSGENCSTNDLSL
8) β AR413-V2R10	CLFRSSLKAYGNGYSSNGTGEQSGYHVEQEKENKLLCHDLP- GTEDFVGHGTVPSDNDIDSGENCSTNDLSLSSSLAKDTSS
9) β AR360-V2R10	CLFRSSLKAYGNGYSSNGTSSSLAKDTSS

B

V2R	NPWYASFSSSVSELSILCCARGRTPPSLGQDESCCTTASSSLAKDTSS
AAA-1	-----AAA-----
AAA-2	-----AAA-----
NTR-1	NPILYNLVSANFQVFLSTLACLCPGWHRKRKRTFSRKPMSMSNHAFSISATRTLY
AAA	-----A-AA-----
AAA	-----AAA-----
OTR	NPWYMLPTGHLFHELIVQFLCCSASVILKGRRLGETSASKNSSSFVLSHRSSQRSCQPSTA
AAA	-----AAAA-----
AAA-1	-----AAA-----
AAA-2	-----AAA-----

Figure 8, Pg. 2

C

SPR
 383X
 355X
 325X
 AATAA
 AATAA

NPILYCLNDRFRLGFKHAFRCCTFISAGDYEGLEMKSTRYLQTOGYVKVRLFTISVVGAEHEPEDPGPKATPSSKLITSCSSRSDSKTWTETSPSSNNVLS

-----X-----X-----X-----AATAA-----AATAA-----

Figure 9

Amino Acid Sequence of the Wild-Type Receptors

A. Amino acid sequence of the wild-type V2R

MLMASTTS~~AV~~PGHPSLPSLPSNSSQERPLDTRDPLLRARAEALLSIVFVAVALSNGLVLAALARRGRRGH~~WAPIHVF~~IGHCLADLAVALFQVLFPQLAWKATDRFRGPDALCRAVKYLQMVGM~~Y~~ASSYMI~~AMTLDRHRAICR~~PMLAYRHGSGAHWNRPVLVAWAFSLLSLPQLFIPAQ~~RNV~~EGSGVTD~~WCACFAEPWGR~~RTYVVTWIALMVFVAPTLGIAACQVLI~~FREIHASL~~VPGPSERPGRRRGRR~~TGSPGEGAHVSA~~AVAKTVRMTLVIVVVYVLCWAPFFLVQLWAAWDPEAPLEGA~~PFVLLMLLASLNSCTNPWI~~YASFSSSVSSELRSLLCC~~ARGRTPPSLGPQ~~DESC~~TTASSSLA~~
KDTSS

(Seq. ID No. 1)

B. Amino acid sequence of the wild-type β_2 AR

MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAIKFERLQTVTNYFITSLACADLMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIELTCVIAVD~~RYFAITSPFKYQ~~SLLTKNKARVILMVIVSGLTSFLPIQMHWYRATHQEAINCYANETCCD~~FFTNQAYAI~~ASSIVSFYVPLVIMVFVYSRVFQEA~~KRQLQKIDK~~SEGRFHVQNLSQVEQDGR~~TGHGLRRSSK~~FCLKEHKALKTLGIIMGTFTLCWLPFFIVNIVHVIQDNLIRKEVYILLN~~WIGYVNSGFNPLI~~YCRSPDFRIAFQELLCLRRSSLKAYGNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTDFVGHQGTVPSDNIDSQGRNCSTNDSSL

(Seq. ID No. 2)

Amino Acid Sequence of the Chimeric Receptors

C. Amino acid sequence of the β_2 AR-V2R chimera (Oakley et al.)

MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAIKFERLQTVTNYFITSLACADLMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIELTCVIAVD~~RYFAITSPFKYQ~~SLLTKNKARVILMVIVSGLTSFLPIQMHWYRATHQEAINCYANETCCD~~FFTNQAYAI~~ASSIVSFYVPLVIMVFVYSRVFQEA~~KRQLQKIDK~~SEGRFHVQNLSQVEQDGR~~TGHGLRRSSK~~FCLKEHKALKTLGIIMGTFTLCWLPFFIVNIVHVIQDNLIRKEVYILLN~~WIGYVNSGFNPLI~~YCRSPDFRIAFQELL~~CARGRTPPSLGPQ~~DESC~~TTASSSLA~~
DTSS

(Seq. ID No. 3)

*shown in bold are the amino acids that were moved to the β_2 AR to increase its affinity for arrestin.

Figure 10**A. Amino acid sequence of the MOR-V2R chimera expressed from the pEArrB-1/MOR vector**

MDSSTGPGNTSDCSDPLAQASCSPAPGSWLNLSHVDGNQSDPCGLNRTGLG
 GNDSLCPQTGSPSMVTAITIMALYSIVCVVGLFGNFLVMYVIVRYTKMKT
 A
 TNIYIFNLALADALATSTLPFQSVNYLMGTWPFGTILCKIVISIDYNNMFT
 SIFTLCTMSVDRIYAVCHPVKALDFRTPRNAKIVNVCNWILSSAIGLPVMF
 MATTKYRQGSIDCTLTFSHPTWYWENLLKICVFIFAFIMPILIIITVCYGLM
 ILRLKSVRMLSGSKEKDRNLRRITRMVLVVAVFIVCWTPIIHIYVIKALI
 TIPETTFQTVSWHFCIALGYTNSCLNPVLYAFLDENFKRCFREFC**AAARGR**
TPPSLGPQDESCTTASSSLAKDTSS

(Seq. ID No. 4)

B. Amino acid sequence of the DIAR-V2R chimera expressed from the pEArrB-1/DIAR vector

MAPNTSTMDEAGLPAERDFSFRIILTACFLSLLILSTLLGNTLVCAAVIRFR
 HLRSKVTNFFVISLAVSDLLVAVLVMPWKAVAEIAGFWPFGSFCNIWVAFD
 IMCSTASILNLCVISVDRYWAISSPFQYERKMTPKAAFILISVAWTLVLI
 SFIPVQLSWHKAKPTWPLDGNFTSLEDTEDDNCDTRLRSRTYAISSSLISFY
 IPVAIMIVTYTISIYRIAQKQIRRIISALERAHAVHAKNCQTAGNGNPVECAQ
 SESSFKMSFKRETKVLKTLSSVIMGVFVCCWLPFFISNCMPVFCGSEETQPF
 CIDSITFDVFWFGWANSSLNPIIYAFNADFQKAFSTLLGCYRLC**AAARGR**
TPPSLGPQDESCTTASSSLAKDTSS

(Seq. ID No. 5)

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C. Amino acid sequence of the 5HT1AR-V2R chimera expressed from the pEArrB-1/5HT1AR vector

MDVLSPGQGNNNTSPAPPFETGGNTTGISDVTVSYQVITSLLLGTLIFCAV
LGNA CVVAAIALERSLQNVANYLIGSLAVTDLMVSVLVLPMAALYQVLNKKW
TLGQVTCDLFIALDVLCTSSILHLCAIALDRYWAITDPIDYVNRKTPRRA
AALISLTWLIGFLISIPMLGWRTPEDRSDPDACTISKDHGYTIYSTFGAF
YIPLLLMLVLYGRIFRAARFRIRKTVKKVEKTGADTRHGASPAQPCKSVN
GESGSRNWRLGVESKAGGALCANGAVRQGGDDGAALVI EVHRVGNSEKHL P
LPSEAGPTPCAPASFERKNERNAEAKRKMALARERKTVKTLGIIMGTFILC
WLPPFFIVALVLPFCESCHMPTLLGAI
INWLGYSNSLLNPVIYAYFNKDFQNAFKKIIKCNFCAAARGRTPPSLGPQD
ESCTTASSSLAKDTSS

(Seq. ID No. 6)

D. Amino acid sequence of the β 3AR-V2R chimera expressed from the pEArrB-1/ β 3AR vector

MAPWPHENSSSLAPWPDLPPLAPNTANTSGLPVGPWEAALAGALLALAVLAT
VGGNLLVIVAIWTPRLQTMNTNVFVTS LAADLVLMGLLVVPPAATLALTGH
WPLGATGCELWTSVDVLCVTAS IETLCALAVDRYLAVTNPLRYGALVTKRC
ARTAVVLVWVSAAVSFAPIMSQWVRVGADAEARCHSNPRCCAFASNMPY
VLLSSSVSFYLP LLVMLFVYARV FVVATRQLRLLRGELGRFPPEESPPAPS
RSLAPAPVGT CAPPEGVPACGRPARLLPLREHRACTLGLIMGTFTLCWL
PFFLANVLRALGGPSLVPGPAFLALNWLGYANSAFNPLIYCRSPDFRSAPR
RLLCRCAAARGRTPPSLGPQDESCTTASSSLAKDTSS

(Seq. ID No. 7)

E. Amino acid sequence of the Edg1R-V2R chimera expressed from the pEArrB-1/Edg1R vector

MGPTSVPLVKAHRSSVSDYVNYDIIVRHNYTGKLNISADKENS IKLTSVV
FILICCFIILENIFVLLTIWTKKFKHRMPYFFIGNLALS DLLAGVAYTANL
LLSGATTYKLT PAQWFLREGSMFVALSASFSLAIAIERYITMLKMKLHN
GSNNFRLLISACWVISLILGGLPIMGWNCISALSSCSTVLP LYHKHYIL
FCTTVFTLLLLSIVILYCRISLVRTSRRLTFRKNTSKASRSSEKSLALL
KTVIIVLSVFIACWAPLFILLLL DVGCKVKTC DILFRAEYFLVLA VLNST
NP I IYTLTNKEMRRAPIRIMSCCKCAAARGRTPPSLGPQDESCTTASSSLA
KDTSS

(Seq. ID No. 8)

Figure 11

A. Nucleotide sequence of the $\beta 2$ AR-V2R chimera

atggggcaaccgagggaacgggcagcgccctcttctgctggcaccacaatagaagccatgcccgggacc
acgacgtcacgcagcaaaaggagcaggggtgtgggtgggtgggcatgggcatcgctcatgtctctcat
cgtcctggccatcggtgttggcaatgtgctgggtcatcacagccattgccaaagtccagagcgtctg
cagacgggtcaccaactacttcatcacttcaactggcctgtgctgatctgggtcatgggcctggcag
tggtgcccttggggccgcccataattcttatgaaaatgtggacttttggcaactctgtgtgcga
gttttggaacttccatttgatgtgctgtgcgtcacggccagcattgagaccctgtgctgtagtcga
gtggatcgctactttggccattacttcaactttcaagtagccagagcctgctgaccaaagaataagg
ccgggtgatcattctgtatgggtgtggattgtgtcaggcccttaacctcttctggccattcagat
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cctggccaaggacacttcatcgtag

(SEQ ID No. 9)

B. Nucleotide sequence of the MOR-V2R chimera

atggacagcagcacccggcccagggaacaccagcgaactgctcagacccttagctcaggcaaggtt
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(SEQ ID No. 10)

C. Nucleotide sequence of the D1AR-V2R chimera

atggctcctaaccacttctaccatggatgaggccgggctgccagcggagaggggatttctccttcc
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(SEQ ID No. 11)

D. Nucleotide sequence of the 5HT1AR-V2R chimera

atggatgtgtctcagccctggtcaggggcaacaacaccacatcaccaccgggctccctttgagaccg
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ccccccagcctgggtccccaaagttagtctctgcaccaccgccagctcctcctctggccaaggac
acttcatcgtga
(SEQ ID No. 12)

Fig. 11, pg. 3

FIGURE 12

**β arr2-GFP Translocation to the MOR and MOR-V2R Chimera
in Response to Morphine**

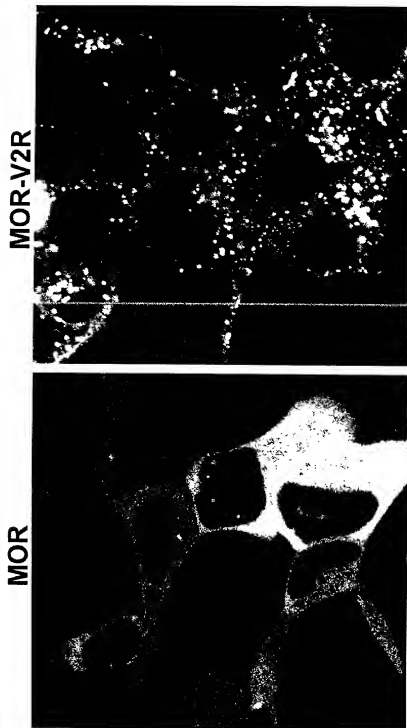


FIGURE 13

**β arr2-GFP Translocation to the D1AR and D1AR-V2R Chimera
in Response to Dopamine**

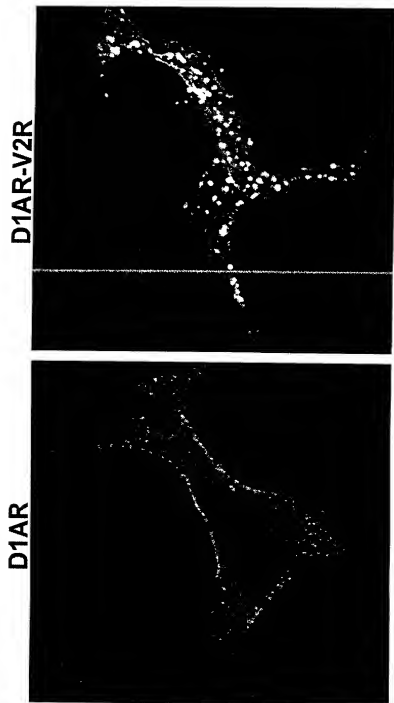


FIGURE 14

β arr2-GFP Translocation to the 5HT1AR and 5HT1AR-V2R
Chimera in Response to Serotonin

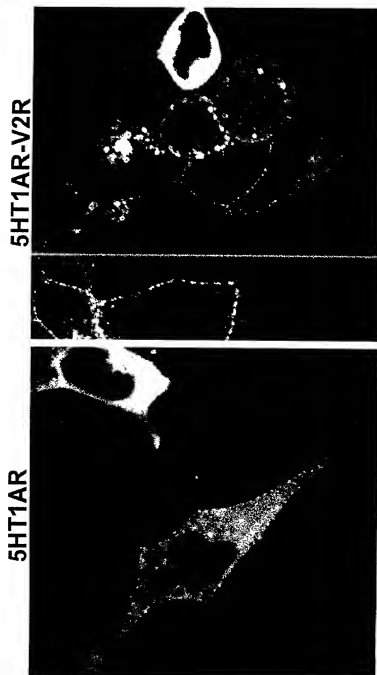


FIGURE 15

β arr2-GFP Translocation to the β_3 AR and β_3 AR-V2R Chimera
 in Response to Isoproterenol

β_3 AR



β_3 AR-V2R

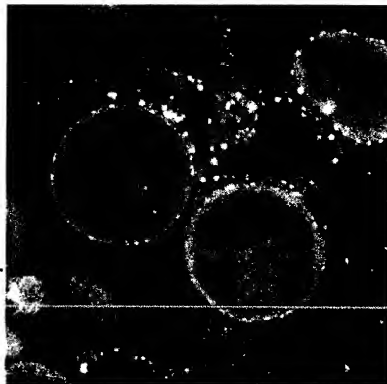


FIGURE 16

β arr2-GFP Translocation to the Edg1 and Edg1-V2R Chimera
in Response to Sphingosine-1-Phosphate

